EUROPEAN SYNCHROTRON RADIATION FACILITY
INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

	Experiment title: Structural insights into energy quenching by the orange carotenoid protein by use of time-resolved WAXS.	**Experiment number**: LS-2734
Beamline: ID09	**Date of experiment**: from: 14 feb 2018 to: 20 Feb 2018	**Date of report**: 02 Mar 2018
Shifts: 18	**Local contact(s)**: KRETZSCHMAR Norman, LEVANTINO Matteo	*Received at ESRF:*

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Report:

Aim of the experiment is to provide time-resolved structural insights into the photoactivation of the Orange Carotenoid Protein (OCP) and to shed light on the timescales of local and global structural changes accompanying the OCP photoactivation. The present study is the first attempt to directly observe the large-scale structural dynamics occurring in OCP solutions upon light activation, so far only hypothesized on the basis of static X-ray patterns.
During the first shifts we characterized the stationary SAXS and WAXS patterns of dark adapted (ground state) OCP (called OCP_O) and of OCP upon continuous illumination at 490 nm to generate the OCP_R state (Figure 1 left panel). We then characterized the timescale of its thermal recovery after photoactivation.
In the second part of the experiment we collected time resolved scattering patterns upon ns laser excitation at 430 nm using the EKSPLA laser available at ID09. However, due to the low quantum yield of OCP photoactivation, only a small fraction of the illuminated sample was activated, resulting in a low S/N ratio in the scattering difference patterns. We then explored the possibility of using the ns pump laser at 527 nm integrated in the Coherent femtosecond system, which generates longer ns pulses (about 500 ns). This approach produced a clear difference signal in the SAXS region, which is in agreement with the static

difference and which evolves in the microsecond to millisecond time scale (Figure 1 right panel). However, we did not have the time to improve the statistics in order to obtain a good S/N ratio and to explore a larger time scale to fully describe the kinetics of OCP structural changes. For these reasons, we applied for a new beamtime that will give us the possibility of completing the study started with the present experiment.

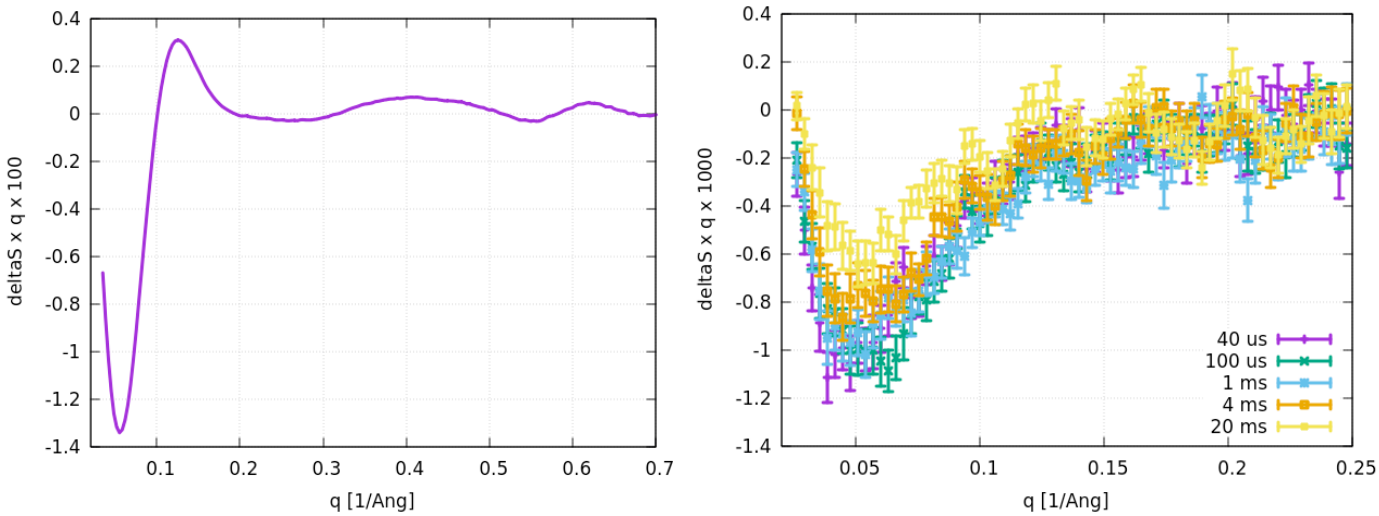

Figure 1. Left panel: OCP_R - OCP_O scattering difference pattern. Right panel: time-resolved scattering difference patterns in the µs to ms time scale after photo-excitation of OCP with a ns laser pulse at 527 nm.